



SEQUENCE LISTING

<110> Kočken, Clemens H.M.
Thomas, Alan W.
Blackman, Michael J.
Withers-Martinez, Chrislaine
Holder, Anthony A.

<120> Efficient expression of Plasmodium apical membrane
antigen 1 in yeast cells

<130> P54200US00

<140> 10/615,615
<141> 2003-07-08

<150> EP 00204697.7
<151> 2000-12-22

<150> PCT/NL01/00934
<151> 2001-12-21

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<170> PatentIn Ver. 2.1

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synthetic gene encoding P. Falciparum AMA-1

ectodomain with *P. pastoris* codon usage

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<223> Description of Artificial Sequence: synthetic gene
encoding *P. Falciparum* AMA-1 ectodomain with *P.*
pastoris codon usage

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1 5 10 15	
tac atg atc aac ttc ggt cgt ggt cag aac tac tgg gag cat cct tac	96
Tyr Met Ile Asn Phe Gly Arg Gly Gln Asn Tyr Trp Glu His Pro Tyr	
20 25 30	
cag aag tct gac gtc tac cat cct atc aac gaa cat agg gag cat cct	144
Gln Lys Ser Asp Val Tyr His Pro Ile Asn Glu His Arg Glu His Pro	
35 40 45	
aag gaa tac gaa tac cca ctg cat caa gag cac act tac cag cag gaa	192
Lys Glu Tyr Glu Tyr Pro Leu His Gln Glu His Thr Tyr Gln Gln Glu	
50 55 60	
gat tct ggt gaa gat gaa aac acc ttg caa cac gct tac ccc atc gat	240
Asp Ser Gly Glu Asp Glu Asn Thr Leu Gln His Ala Tyr Pro Ile Asp	
65 70 75 80	
cat gaa gga gct gaa cca gcc cct cag gaa caa aac ttg ttc tct tcc	288
His Glu Gly Ala Glu Pro Ala Pro Gln Glu Gln Asn Leu Phe Ser Ser	
85 90 95	
atc gaa atc gtg gaa aga tcc aac tac atg ggt aac cca tgg act gag	336
Ile Glu Ile Val Glu Arg Ser Asn Tyr Met Gly Asn Pro Trp Thr Glu	
100 105 110	
tac atg gca aag tac gac atc gag gaa gtg cac gga agt ggt atc agg	384
Tyr Met Ala Lys Tyr Asp Ile Glu Glu Val His Gly Ser Gly Ile Arg	
115 120 125	
gtt gat ctg ggt gaa gat gcc gaa gtc gct ggt act cag tac aga ctc	432
Val Asp Leu Gly Glu Asp Ala Glu Val Ala Gly Thr Gln Tyr Arg Leu	
130 135 140	
cct tct ggt aag tgc cct gtt ttc gga aag ggt atc atc atc gaa aac	480
Pro Ser Gly Lys Cys Pro Val Phe Gly Lys Gly Ile Ile Ile Glu Asn	
145 150 155 160	
tct aag act act ttc ctc aag cct gtt gct act ggt aac caa gat ctt	528
Ser Lys Thr Thr Phe Leu Lys Pro Val Ala Thr Gly Asn Gln Asp Leu	
165 170 175	
aag gac gga ggt ttc gct ttc cca cct act aac cct ctg atc tct cca	576

Lys	Asp	Gly	Gly	Phe	Ala	Phe	Pro	Pro	Thr	Asn	Pro	Leu	Ile	Ser	Pro		
			180					185					190				
atg	act	ttg	aac	ggt	atg	cgt	gac	ttc	tac	aag	aac	aac	gaa	tac	gtc	624	
Met	Thr	Leu	Asn	Gly	Met	Arg	Asp	Phe	Tyr	Lys	Asn	Asn	Glu	Tyr	Val		
		195					200					205					
aag	aac	ttg	gat	gaa	ttg	act	ttg	tgt	agt	aga	cac	gct	gga	aac	atg	672	
Lys	Asn	Leu	Asp	Glu	Leu	Thr	Leu	Cys	Ser	Arg	His	Ala	Gly	Asn	Met		
	210					215					220						
aac	cct	gat	aac	gac	aag	aac	agt	aac	tac	aag	tac	ccc	gcg	gtt	tac	720	
Asn	Pro	Asp	Asn	Asp	Lys	Asn	Ser	Asn	Tyr	Lys	Tyr	Pro	Ala	Val	Tyr		
225					230					235				240			
gac	tac	aac	gat	aag	aag	tgt	cac	atc	ctg	tac	atc	gct	gcc	caa	gaa	768	
Asp	Tyr	Asn	Asp	Lys	Lys	Cys	His	Ile	Leu	Tyr	Ile	Ala	Ala	Gln	Glu		
				245					250					255			
aac	aac	gga	cca	aga	tac	tgt	aac	aag	gat	caa	agt	aag	aga	aac	tct	816	
Asn	Asn	Gly	Pro	Arg	Tyr	Cys	Asn	Lys	Asp	Gln	Ser	Lys	Arg	Asn	Ser		
			260					265					270				
atg	ttc	tgt	ttc	aga	cct	gca	aag	gac	aag	ctg	ttc	gaa	aac	tac	gtg	864	
Met	Phe	Cys	Phe	Arg	Pro	Ala	Lys	Asp	Lys	Leu	Phe	Glu	Asn	Tyr	Val		
		275					280					285					
tac	ttg	tcc	aag	aac	gtt	gtc	gat	aac	tgg	gaa	gaa	gtc	tgc	cca	aga	912	
Tyr	Leu	Ser	Lys	Asn	Val	Val	Asp	Asn	Trp	Glu	Glu	Val	Cys	Pro	Arg		
	290					295					300						
aag	aac	ctc	gag	aac	gca	aag	ttc	ggt	ctg	tgg	gtc	gat	ggt	aac	tgt	960	
Lys	Asn	Leu	Glu	Asn	Ala	Lys	Phe	Gly	Leu	Trp	Val	Asp	Gly	Asn	Cys		
305					310					315					320		
gaa	gac	atc	cct	cat	gtg	aac	gag	ttc	agt	gct	aac	gat	ttg	ttc	gag	1008	
Glu	Asp	Ile	Pro	His	Val	Asn	Glu	Phe	Ser	Ala	Asn	Asp	Leu	Phe	Glu		
				325					330					335			
tgt	aac	aag	ctg	gtc	ttc	gag	ttg	tct	gcc	agt	gac	caa	cct	aag	cag	1056	
Cys	Asn	Lys	Leu	Val	Phe	Glu	Leu	Ser	Ala	Ser	Asp	Gln	Pro	Lys	Gln		
			340					345					350				
tac	gaa	cag	cat	ttg	act	gac	tac	gaa	aag	atc	aag	gaa	gga	ttc	aag	1104	
Tyr	Glu	Gln	His	Leu	Thr	Asp	Tyr	Glu	Lys	Ile	Lys	Glu	Gly	Phe	Lys		
		355					360					365					
aac	aag	aac	gcc	gat	atg	atc	aag	tcc	gct	ttc	ctc	cca	acc	ggt	gca	1152	
Asn	Lys	Asn	Ala	Asp	Met	Ile	Lys	Ser	Ala	Phe	Leu	Pro	Thr	Gly	Ala		
	370					375					380						
ttc	aaa	gca	gat	aga	tac	aag	tct	cac	ggt	aag	ggt	tac	aac	tgg	gga	1200	
Phe	Lys	Ala	Asp	Arg	Tyr	Lys	Ser	His	Gly	Lys	Gly	Tyr	Asn	Trp	Gly		
385					390					395				400			
aac	tac	aac	aga	gaa	acc	caa	aag	tgt	gaa	atc	ttc	aac	gtc	aag	cct	1248	
Asn	Tyr	Asn	Arg	Glu	Thr	Gln	Lys	Cys	Glu	Ile	Phe	Asn	Val	Lys	Pro		

	405	410	415	
acc tgc ctc atc aac gac aag tcc tac att gcg act act gcc ctg tct				1296
Thr Cys Leu Ile Asn Asp Lys Ser Tyr Ile Ala Thr Thr Ala Leu Ser				
	420	425	430	
cat cca atc gaa gtc gaa cac aac ttc ccc tgc agt ctc tac aag gac				1344
His Pro Ile Glu Val Glu His Asn Phe Pro Cys Ser Leu Tyr Lys Asp				
	435	440	445	
gag atc aag aag gaa atc gag cgt gaa agt aag cgt atc aag ttg aac				1392
Glu Ile Lys Lys Glu Ile Glu Arg Glu Ser Lys Arg Ile Lys Leu Asn				
	450	455	460	
gat aac gac gac gaa ggt aac aag aag atc atc gca cct agg atc ttc				1440
Asp Asn Asp Asp Glu Gly Asn Lys Lys Ile Ile Ala Pro Arg Ile Phe				
	465	470	475	480
atc tcc gat gac aag gat tcc ctc aag tgt cct tgt gac cct gag atg				1488
Ile Ser Asp Asp Lys Asp Ser Leu Lys Cys Pro Cys Asp Pro Glu Met				
	485	490	495	
gtg agt cag tcc act tgt aga ttc ttc gtt tgc aag tgc gtc gaa cgt				1536
Val Ser Gln Ser Thr Cys Arg Phe Phe Val Cys Lys Cys Val Glu Arg				
	500	505	510	
aga gcc gaa gtc act agt aac aac gaa gtt gtc gtg aag gaa gaa tac				1584
Arg Ala Glu Val Thr Ser Asn Asn Glu Val Val Val Lys Glu Glu Tyr				
	515	520	525	
aag gat gaa tac gct gat att cca gag cat aag cct acg tac gat aac				1632
Lys Asp Glu Tyr Ala Asp Ile Pro Glu His Lys Pro Thr Tyr Asp Asn				
	530	535	540	
atg aag atc atc atc gct agt tct gct gct gtc gct gtt ctg gct act				1680
Met Lys Ile Ile Ile Ala Ser Ser Ala Ala Val Ala Val Leu Ala Thr				
	545	550	555	560
atc ctc atg gtg tac ctt tac aag aga aag gga aac gct gag aag tac				1728
Ile Leu Met Val Tyr Leu Tyr Lys Arg Lys Gly Asn Ala Glu Lys Tyr				
	565	570	575	
gac aag atg gat caa cct caa cat tac ggt aag agt act tcc agg aac				1776
Asp Lys Met Asp Gln Pro Gln His Tyr Gly Lys Ser Thr Ser Arg Asn				
	580	585	590	
gat gag atg ttg gat cca gag gcc tcc ttc tgg ggt gag gag aag aga				1824
Asp Glu Met Leu Asp Pro Glu Ala Ser Phe Trp Gly Glu Glu Lys Arg				
	595	600	605	
gcc tct cat act act cca gtt ttg atg gag aag cct tac tac taa				1869
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	610	615	620	

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 <223> Description of Artificial Sequence: synthetic gene
 encoding P. Falciparum AMA-1 ectodomain with P.
 pastoris codon usage

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Tyr Met Ile Asn Phe Gly Arg Gly Gln Asn Tyr Trp Glu His Pro Tyr
          20           25           30
Gln Lys Ser Asp Val Tyr His Pro Ile Asn Glu His Arg Glu His Pro
          35           40           45
Lys Glu Tyr Glu Tyr Pro Leu His Gln Glu His Thr Tyr Gln Gln Glu
          50           55           60
Asp Ser Gly Glu Asp Glu Asn Thr Leu Gln His Ala Tyr Pro Ile Asp
          65           70           75           80
His Glu Gly Ala Glu Pro Ala Pro Gln Glu Gln Asn Leu Phe Ser Ser
          85           90           95
Ile Glu Ile Val Glu Arg Ser Asn Tyr Met Gly Asn Pro Trp Thr Glu
          100          105          110
Tyr Met Ala Lys Tyr Asp Ile Glu Glu Val His Gly Ser Gly Ile Arg
          115          120          125
Val Asp Leu Gly Glu Asp Ala Glu Val Ala Gly Thr Gln Tyr Arg Leu
          130          135          140
Pro Ser Gly Lys Cys Pro Val Phe Gly Lys Gly Ile Ile Ile Glu Asn
          145          150          155          160
Ser Lys Thr Thr Phe Leu Lys Pro Val Ala Thr Gly Asn Gln Asp Leu
          165          170          175
Lys Asp Gly Gly Phe Ala Phe Pro Pro Thr Asn Pro Leu Ile Ser Pro
          180          185          190
Met Thr Leu Asn Gly Met Arg Asp Phe Tyr Lys Asn Asn Glu Tyr Val
          195          200          205
Lys Asn Leu Asp Glu Leu Thr Leu Cys Ser Arg His Ala Gly Asn Met
          210          215          220
Asn Pro Asp Asn Asp Lys Asn Ser Asn Tyr Lys Tyr Pro Ala Val Tyr
          225          230          235          240
Asp Tyr Asn Asp Lys Lys Cys His Ile Leu Tyr Ile Ala Ala Gln Glu
          245          250          255
Asn Asn Gly Pro Arg Tyr Cys Asn Lys Asp Gln Ser Lys Arg Asn Ser
          260          265          270

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Met	Phe	Cys	Phe	Arg	Pro	Ala	Lys	Asp	Lys	Leu	Phe	Glu	Asn	Tyr	Val	
		275					280					285				
Tyr	Leu	Ser	Lys	Asn	Val	Val	Asp	Asn	Trp	Glu	Glu	Val	Cys	Pro	Arg	
	290					295					300					
Lys	Asn	Leu	Glu	Asn	Ala	Lys	Phe	Gly	Leu	Trp	Val	Asp	Gly	Asn	Cys	
305					310					315					320	
Glu	Asp	Ile	Pro	His	Val	Asn	Glu	Phe	Ser	Ala	Asn	Asp	Leu	Phe	Glu	
				325					330					335		
Cys	Asn	Lys	Leu	Val	Phe	Glu	Leu	Ser	Ala	Ser	Asp	Gln	Pro	Lys	Gln	
			340					345					350			
Tyr	Glu	Gln	His	Leu	Thr	Asp	Tyr	Glu	Lys	Ile	Lys	Glu	Gly	Phe	Lys	
		355					360					365				
Asn	Lys	Asn	Ala	Asp	Met	Ile	Lys	Ser	Ala	Phe	Leu	Pro	Thr	Gly	Ala	
	370					375					380					
Phe	Lys	Ala	Asp	Arg	Tyr	Lys	Ser	His	Gly	Lys	Gly	Tyr	Asn	Trp	Gly	
385					390					395					400	
Asn	Tyr	Asn	Arg	Glu	Thr	Gln	Lys	Cys	Glu	Ile	Phe	Asn	Val	Lys	Pro	
				405					410					415		
Thr	Cys	Leu	Ile	Asn	Asp	Lys	Ser	Tyr	Ile	Ala	Thr	Thr	Ala	Leu	Ser	
			420					425					430			
His	Pro	Ile	Glu	Val	Glu	His	Asn	Phe	Pro	Cys	Ser	Leu	Tyr	Lys	Asp	
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Glu	Ile	Lys	Lys	Glu	Ile	Glu	Arg	Glu	Ser	Lys	Arg	Ile	Lys	Leu	Asn	
	450					455					460					
Asp	Asn	Asp	Asp	Glu	Gly	Asn	Lys	Lys	Ile	Ile	Ala	Pro	Arg	Ile	Phe	
465					470					475					480	
Ile	Ser	Asp	Asp	Lys	Asp	Ser	Leu	Lys	Cys	Pro	Cys	Asp	Pro	Glu	Met	
				485					490					495		
Val	Ser	Gln	Ser	Thr	Cys	Arg	Phe	Phe	Val	Cys	Lys	Cys	Val	Glu	Arg	
			500					505					510			
Arg	Ala	Glu	Val	Thr	Ser	Asn	Asn	Glu	Val	Val	Val	Lys	Glu	Glu	Tyr	
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	530					535					540					
Met	Lys	Ile	Ile	Ile	Ala	Ser	Ser	Ala	Ala	Val	Ala	Val	Leu	Ala	Thr	
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Ile	Leu	Met	Val	Tyr	Leu	Tyr	Lys	Arg	Lys	Gly	Asn	Ala	Glu	Lys	Tyr	
				565					570					575		

Asp Lys Met Asp Gln Pro Gln His Tyr Gly Lys Ser Thr Ser Arg Asn
580 585 590

Asp Glu Met Leu Asp Pro Glu Ala Ser Phe Trp Gly Glu Glu Lys Arg
595 600 605

Ala Ser His Thr Thr Pro Val Leu Met Glu Lys Pro Tyr Tyr
610 615 620